Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Idiotypes in Medicine: Autoimmunity, Infection and Cancer
Y. Shoenfeld, R.C. Kennedy and S. Ferrone, editors.

X-RAY CRYSTALLOGRAPHIC STUDIES OF THE IDIOTYPIC CASCADE

Nenad Ban and Alexander McPherson

Department of Biochemistry, University of California, Riverside, CA 92521, USA

Idiotypes are the sum of idiotopes or serologically specified antigenic determinants unique to an antibody or group of antibodies. The demonstration that an antibody made in response to the original antigen can itself become an antigen and elicit the synthesis of a secondary antibody (Rodkey, 1974) led to the formulation of a hypothesis by Lindenmann and Jerne (Lindenmann, 1973; Jerne, 1974) that the immune system responds to foreign substances as a regulatory network composed of idiotypes (Ab1s) and their anti-idiotypes (Ab2s) (for reviews see Greene and Nisonoff, 1984; Davie et al., 1986). The potential regulatory role of idiotype–anti-idiotype (Id–anti-Id) interactions has since been the object of numerous studies (reviewed in Greene and Nisonoff, 1984; Gaulton and Greene, 1986). This response can be divided into an antigen non-inhibitable (Ab2α) and an antigen-inhibitable group (Ab2β). A third group, which is antigen-inhibitable because of steric hindrance with the antigen binding site, is designated Ab2γ (reviewed in Dalgleish and Kennedy, 1988).

Anti-idiotypic antibodies produced against the combining site idiotope may carry an "internal image" of the external antigen and are also known as internal image antibodies. A true internal image can be differentiated further from Ab2γ by direct visualization of interacting molecules or by the fact that only Ab2β is able to induce an Ab1-like anti-anti-idiotypic (Ab3) response. Internal image molecules, stereochemically complementary to the surface of the Ab1 combining site, can even induce immune mediated responses similar to the original antigen, and this has, in fact, been used to produce vaccines (reviewed in Williams et al., 1990; Poskit et al., 1991). As an example, Ab2β anti-Ids have been developed against different:

1. viral: type B viral hepatitis (Kennedy et al., 1986), the rabies virus glycoprotein (Reagan et al., 1983), polio virus type 2 (Fons et al., 1985), influenza hemagglutinin (Anders *et al.*, 1989), and bluetonge virus (Grieder et al., 1990);
2. bacterial: *Streptococcus pneumoniae* (McNamara et al., 1984), *Pseudomonas aeruginosae* (Schrieber et al., 1991);
3. parasitic: *Trypanosoma rhodesiense* (Sacks et al., 1982), *Schistosomias masoni* (Kresina and Olds, 1989; Velge-Roussel et al., 1989);
4. fungal metabolites (which represent major agricultural contamination problems): trichothecene mycotoxin T-2 (Chanh et al., 1990); and
5. tumor antigens – with potential use in cancer therapy (reviewed in Langone, 1989).

Furthermore, this phenomenon has been utilized to identify putative receptors for the import of proteins into mitochondria (Pain et al., 1990), and anti-anti-IgE idiotypic antibodies have been shown to mimic IgE in their binding to Fc_ε receptor on mast cells involved in complex allergic responses (Baniyash and Eshhar, 1987).

These results suggest that there may exist significant structural mimicry between the "complementarity determining regions" (CDRs) of internal image Ab2s and the original antigen. This represents one of the most interesting areas of structure–function relations, and several structural studies dealt with this unique problem. Since X-ray crystallography is currently the only technique capable of solving this problem on a molecular level, in this chapter, we will try to summarize the results obtained by crystallographic analysis of components of the idiotypic cascade.

Structural studies of idiotypic cascades have been

carried out using exclusively antibody fragments (reviewed in Mariuzza and Poljak, 1993; Pan et al., 1995). This is because intact antibodies are large and flexible molecules which are rather difficult to crystallize (Harris et al., 1992) (Figure 1, opposite). Single crystal X-ray diffraction studies have shown that antibody Fab fragments are multimeric proteins consisting of light (L) and heavy (H) polypeptide chains appearing as four homologous globular domains, organized in pairs, that share a common 3-D arrangement. The "immunoglobulin" fold consists of two antiparallel β-sheets formed by three and four antiparallel strands in the constant light (C_L) and heavy (C_H1) domains, and five and four antiparallel strands in variable light (V_L) and heavy (V_H) domains. These are connected by loops showing a conserved topology (for reviews, see Amzel and Poljak, 1979; Davies and Metzger, 1983; Alzari et al., 1988; Davies et al., 1990). The specificity of immunoglobulins is determined by the amino acid sequences of three hypervariable loops of both the heavy and the light chains of a variable domain. These CDRs occur at the extremities of the molecule, fully exposed to solvent, where they form the antigen binding site. Using the techniques of molecular biology it is also possible to produce, by expression in bacteria, only V_H–V_L domain pairs, called Fv. Utilizing this system it is possible to perform site directed mutagenesis, and selectively change amino acids forming CDRs, and monitor the binding capabilities of new antibody combining regions.

In order to study the relationship between an anti-idiotypic antibody and the original antigen it is necessary to know the structure of both on a molecular level, and preferably to know the details of interactions between idiotype and each of these molecules. This, on the other hand would require two different complexes to be crystallized and their structures determined. This illustrates the difficulty associated with this type of study. To date, there have been five different investigations dealing with anti-idiotypes. These involved two different studies of an anti-lysozyme idiotypic system (Bentley et al., 1990; Fields et al., 1995), an anti-angiotensin II system (Garcia et al., 1992), an anti-feline infectious peritonitis virus (FIPV) system (Ban et al., 1994), an anti-lipopolysaccharide A antigen of *Brucella abortus* system (Evans et al., 1994), and an anti-anti-idiotypic Fab fragment belonging to the high molecular weight-melanoma associated antigen (HMW-MAA) idiotypic cascade (Ban et al., 1996).

ANTI-LYSOZYME SYSTEM

FabD1.3(Ab1)–FabE225(Ab2) and FabD1.3–Lysozyme Complex

The first crystallographic analysis of the idiotope–anti-idiotope (Id–anti-Id) phenomena on a molecular level was achieved by Poljak and co-workers (Bentley et al., 1990). This study included solving the structure of a complex between a Fab fragment of D1.3 antibody in complex with lysozyme, and the structure of a complex between the Fab fragment of idiotypic D1.3 and a Fab fragment of an anti-idiotypic antibody E225. Anti-idiotypic antibody E225 was shown to be an Ab2β, carrying an internal image of an external antigen. The structure of the Id–anti-Id complex was solved at 2.5 Å.

Two molecules forming a complex are approximately aligned along their long axis. The interaction between the idiotype and anti-idiotype is formed primarily through hypervariable regions. There are 13 residues on five CDRs of the D1.3 that interact with fourteen residues on six CDRs of the anti-idiotypic E225. There is also one framework residue on the V_L domain of each molecule that contributes to the binding interactions. Both the Fab of the anti-idiotypic E225 and the Fab of the idiotypic D1.3 are centered on V_L domains of the complementary molecule in the complex. In spite of this interaction involving primarily V_L domains, the V_H domain on the anti-Id is responsible for 45% of the contacts. There are nine hydrogen bonds formed upon complex formation, and one salt link between E225 Arg ^{L30} and D1.3 Asp^{H54} (summarized in Table 1.A.).

The paratope of D1.3 (lysozyme binding site) consists of 13 residues. Of these 13 residues, seven are in common with the residues recognized by the anti-idiotype E225 (Table 1.A.). Comparison of the CDRs of the idiotypic D1.3 in the Id–anti-Id complex and the complex between Fab, or Fv with the antigen lysozyme, showed that there are significant side chain conformational changes. This is probably the result of different steric requirements for binding of the lysozyme versus the anti-idiotype by the idiotopic D1.3. Interestingly, there is no conformational change of the side chains when free D1.3 idiotope is compared with that bound to the antigen (Bhat et al., 1990).

This structure did not provide a molecular explanation for the mechanism of anti-idiotypic mimicry. A detailed comparison of the nature of the interactions within the lysozyme-D1.3 and the D1.3–E225 complex showed them to be quite different. There are

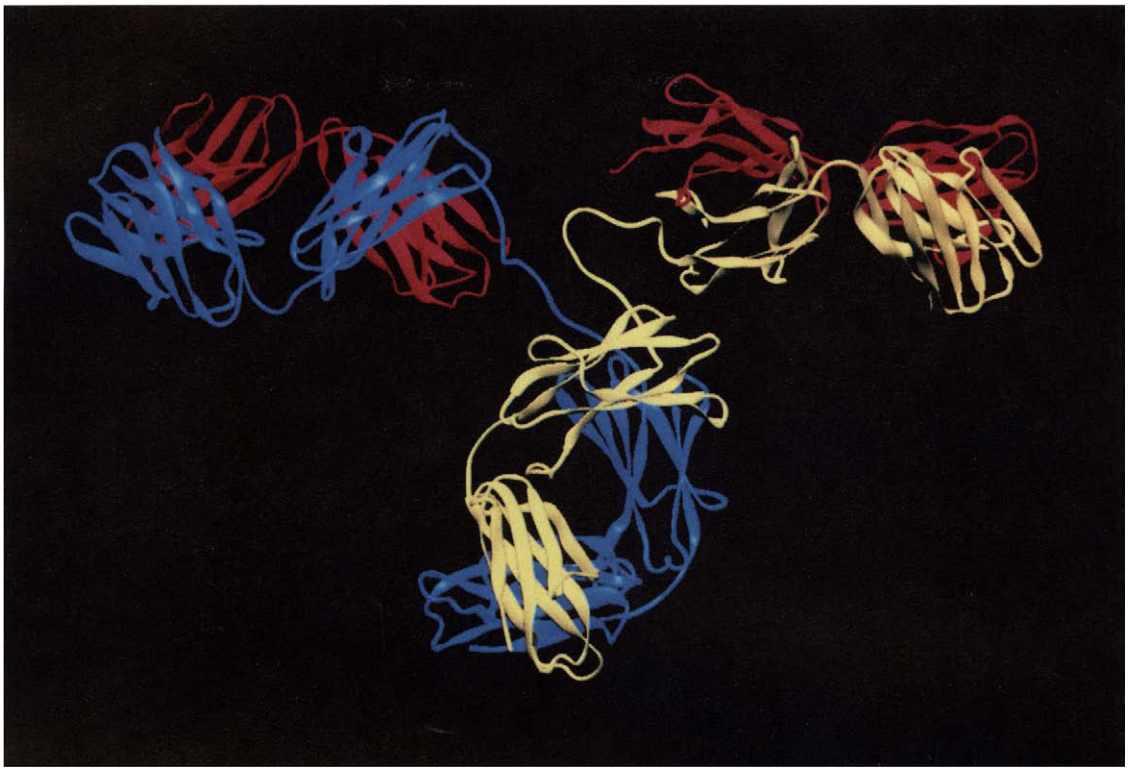


Figure 1. Ribbon representation of the structure of the murine antibody against canine lymphoma determined by X-ray analysis of the triclinic crystals. The heavy chains are shown in yellow and blue, while the light chains are in red. The Fc stem of the molecule projects towards the viewer and assumes an asymmetric, oblique orientation with respect to the Fabs. This orientation illustrates the vast difference in hinge angles of about 65° and 115°. One of the Fabs is viewed along the axis through the switch peptides. This Fab has an elbow angle of 143°, in contrast to the other which has an elbow of 159°.

several plausible explanations for this:

a) there are structural differences that occur at the combining site of the idiotopic D1.3;
b) the open loop structure of anti-idiotypic antibody's CDRs may not be able to mimic the partly α-helical conformation of the lysozyme epitope recognized by the idiotopic D1.3; and,
c) the potential for anti-idiotopic mimicry is reduced because of only partial overlap between the paratope and the idiotope of D1.3.

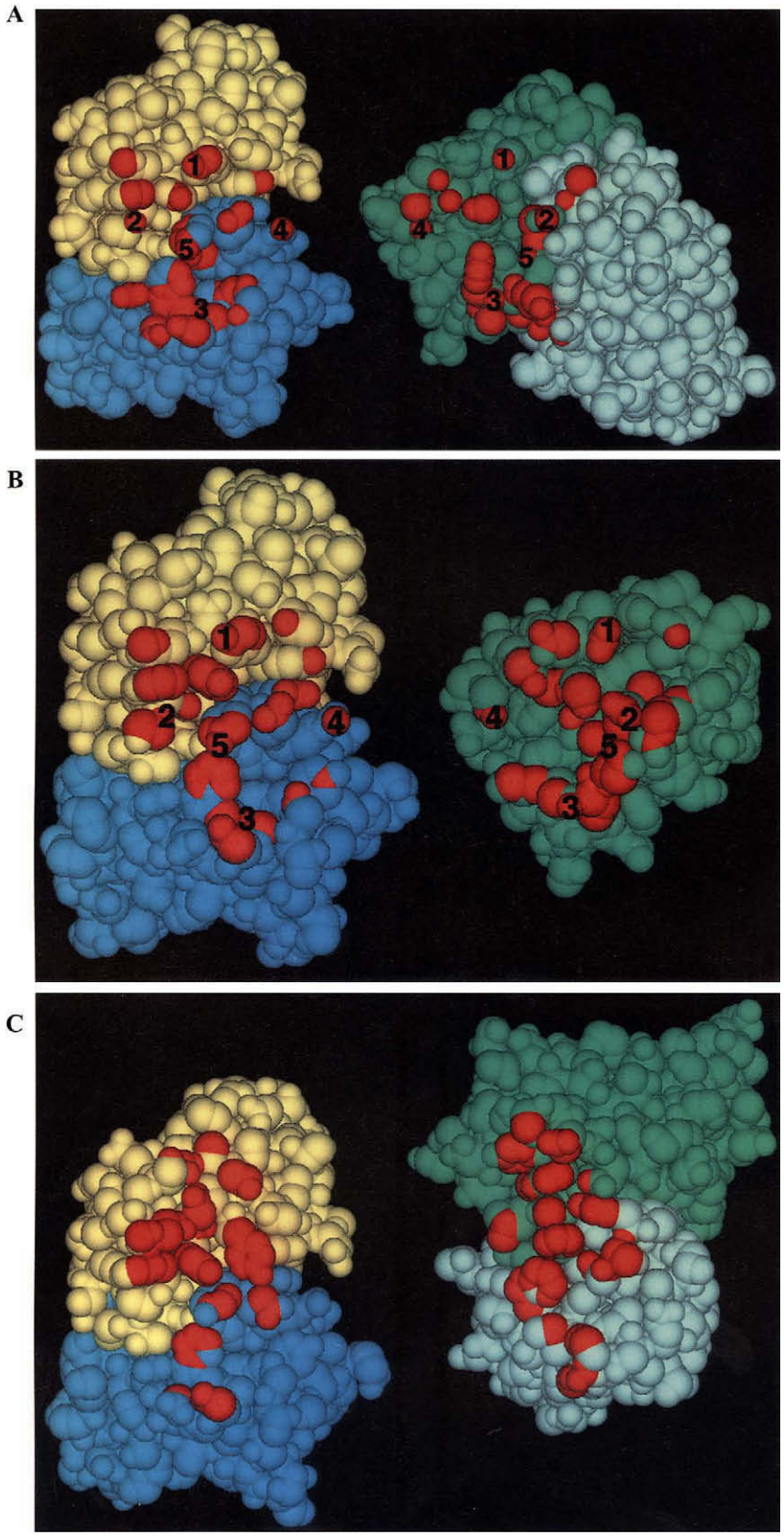
In addition, anti-idiotopic antibody E225 has a considerably lower affinity for D1.3 (2.0×10^5/M) than the original lysozyme antigen (1.4×10^9/M).

FvD1.3(Ab1)–FvE5.2(Ab2) and FabD1.3–Lysozyme complex

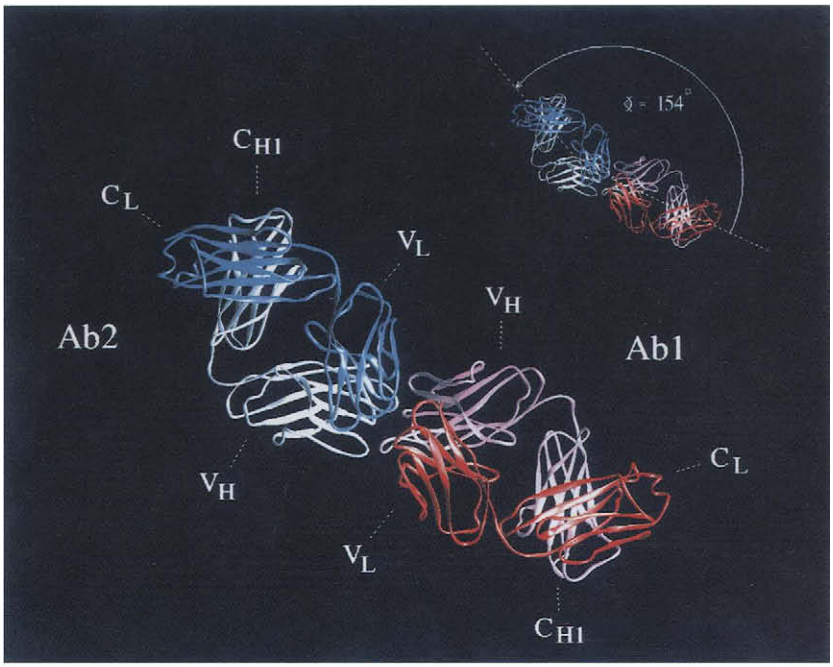
Another very interesting study on the molecular basis of antigen mimicry was completed using again an anti-lysozyme idotypic cascade (Fields et al., 1995). This analysis was the first to visualize molecular mimicry of the external antigen (lysozyme) by an anti-idiotypic

---

Figure 2 (*overleaf*). Comparison of Id–anti-Id and antigen–antibody interactions. (**A**) Contacting atoms (in red) in the D1.3–E5.2 complex. V_L D1.3 I in yellow, V_H blue, V_L E5.2 light green, V_H green. Residues of D1.3 that contact E5.2 are: V_L His 30, Tyr 32, Tyr 49, Tyr 50, Trp 92 and V_H Thr 30, Gly 31, Tyr 32, Gly 33, Trp 52, Gly 53, Asp 54, Asn 56, Asp 58, Glu 98, Arg 99, Asp 100, Tyr 101; numbers 1–5 correspond to atoms in Table 1. (**B**) D1.3 atoms involved in contacts with lysozyme (compare with A). Residues of D1.3 that contact lysozyme in V_L are: His 30, Tyr 32, Tyr 49, Tyr 50, Thr 53, Phe 91, Trp 92, Ser 93; and in V_H: Gly 31, Tyr 32, Trp 52, Gly 53, Asp 54, Arg 99, Asp 100, Tyr 101, Arg 102. Lysozyme is shown in green; atoms numbered 1–5 are listed in Table 1. (**C**) Contacting atoms of D1.3 (left) and the anti-idiotope E225 (right). In this Id–anti-Id complex the D1.3 side chains (VL, Tyr 50, Trp 92 and V_H, Asp 100) have changed conformations to give a very different combining structure from that shown in A and B. In E225, V_L is light green, V_H is green, and contacting atoms are red. (Reproduced with permission from Fields et al, 1995, courtesy of Drs. Poljak and Mariuzza.)


A
1
2
5
4
3
1
4
2
5
3
B
1
2
5
4
3
1
4
2
5
3
C

A



B

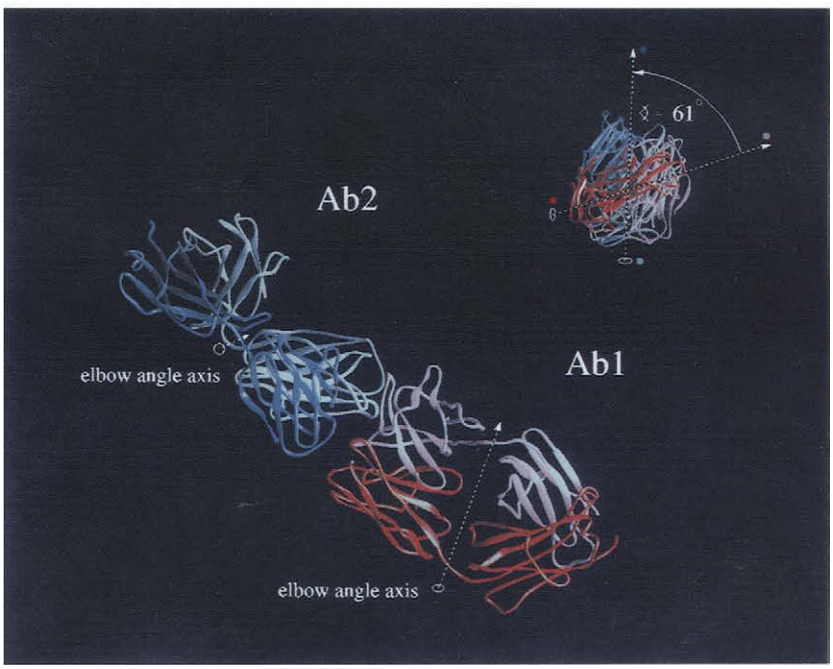


Figure 3. Structure of the 730.1.4–409.5.3 (Ab1–Ab2) complex. (**A**) Anti-idiotypic Fab (labeled Ab2) is in light (heavy chain) and dark blue (light chain). Idiotypic Fab (labeled Ab1) is in pink (heavy chain) and red (light chain). The pseudo 2-fold axis relating VL and VH domains of one Fab forms a 154° angle with the axis, relating equivalent domains of the other Fab. This is schematically represented in the inset. (**B**) A second view of the complex. Elbow angle axis vectors are indicated on Fab of the Ab1 and Fab of the Ab2. Relative rotation of two Fabs, with respect to each other, around the approximate long axis of the complex is 61°, as shown in the inset. This angle was calculated by projecting two elbow angle axes onto the plane perpendicular to the long axis of the molecule. Axes for each Fab were established by the coordinates of two carbon α atoms at the center of switch peptides.

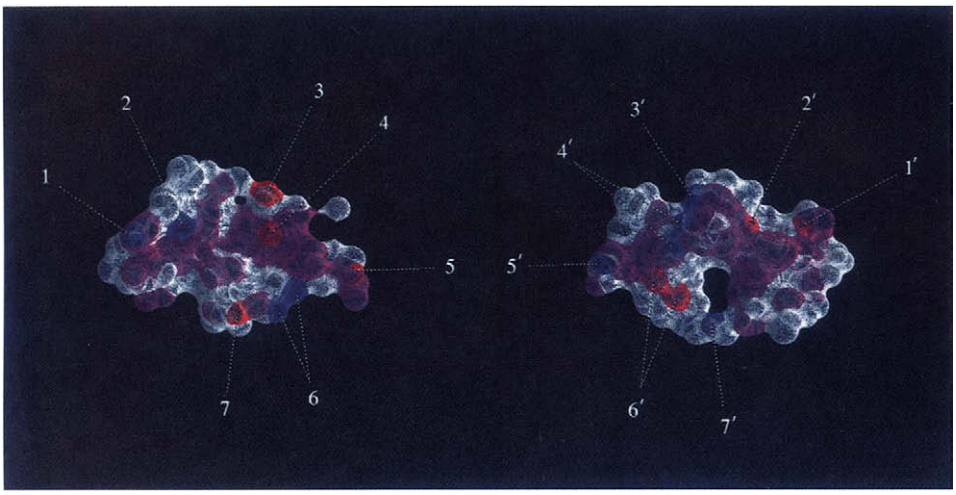


Figure 4. Accessible surface area of the interacting region on the Fab fragment of the idiotope (730.1.4) is shown on the left (**A**) and of the Fab fragment of the anti-idiotope (409.5.3) on the right (**B**). This region is buried upon complex formation. Surfaces of atoms involved in van der Waals contacts are colored purple. Hydrogen bond donors are displayed in blue and hydrogen bond acceptors are shown in red. Eight groups involved in hydrogen bonding are labeled to facilitate identification of contact points between idiotope and anti-idiotope. The atoms on the idiotope are labeled clockwise with numbers 1 to 8, and those on the anti-idiotope counterclockwise with numbers 1' to 8'.

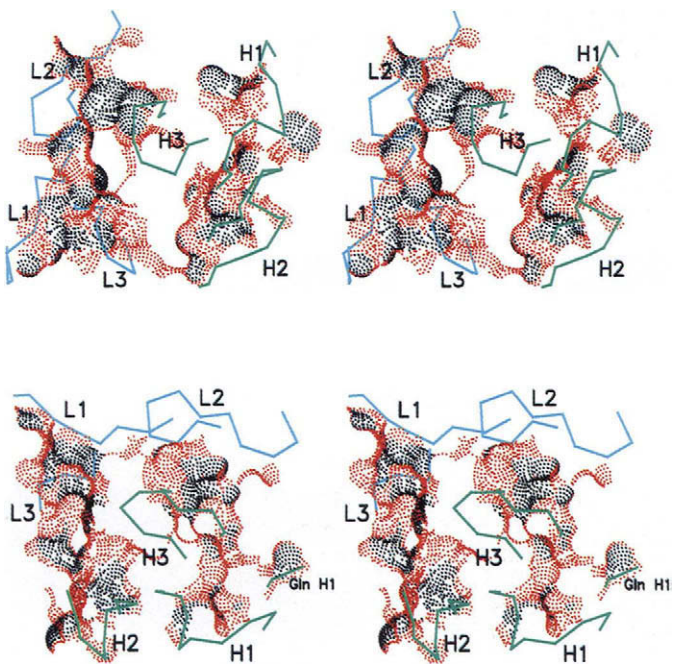


Figure 6. Stereoview of the solvent-excluded-molecular surfaces of both Fabs in the Ab1–Ab2 Fab complex, positioned with the complex viewed down its long axis and holding stationary from the Fab from Ab1 (YsT9.1, top) such that its surface was visible and rotating the Fab from Ab2 (T91AJ5, bottom) by approximately 180° about the horizontal figure axis and positioning it below the idiotope Fab. Those regions of the solvent-excluded surface of one Fab that approach within 0.3 Å of the surface on the other Fab are colored black, and the remainder of the solvent excluded surface is red. The six hypervariable loops of each Fab are indicated with the light chain in blue and heavy chain in green. The two Fabs are observed to be rotated by approximately 90° to each other about the long axis of the complex, and each Fab displays two footprints where it is contacted by the other Fab. (Reproduced with permission from Evans et al., 1994.)

A

B

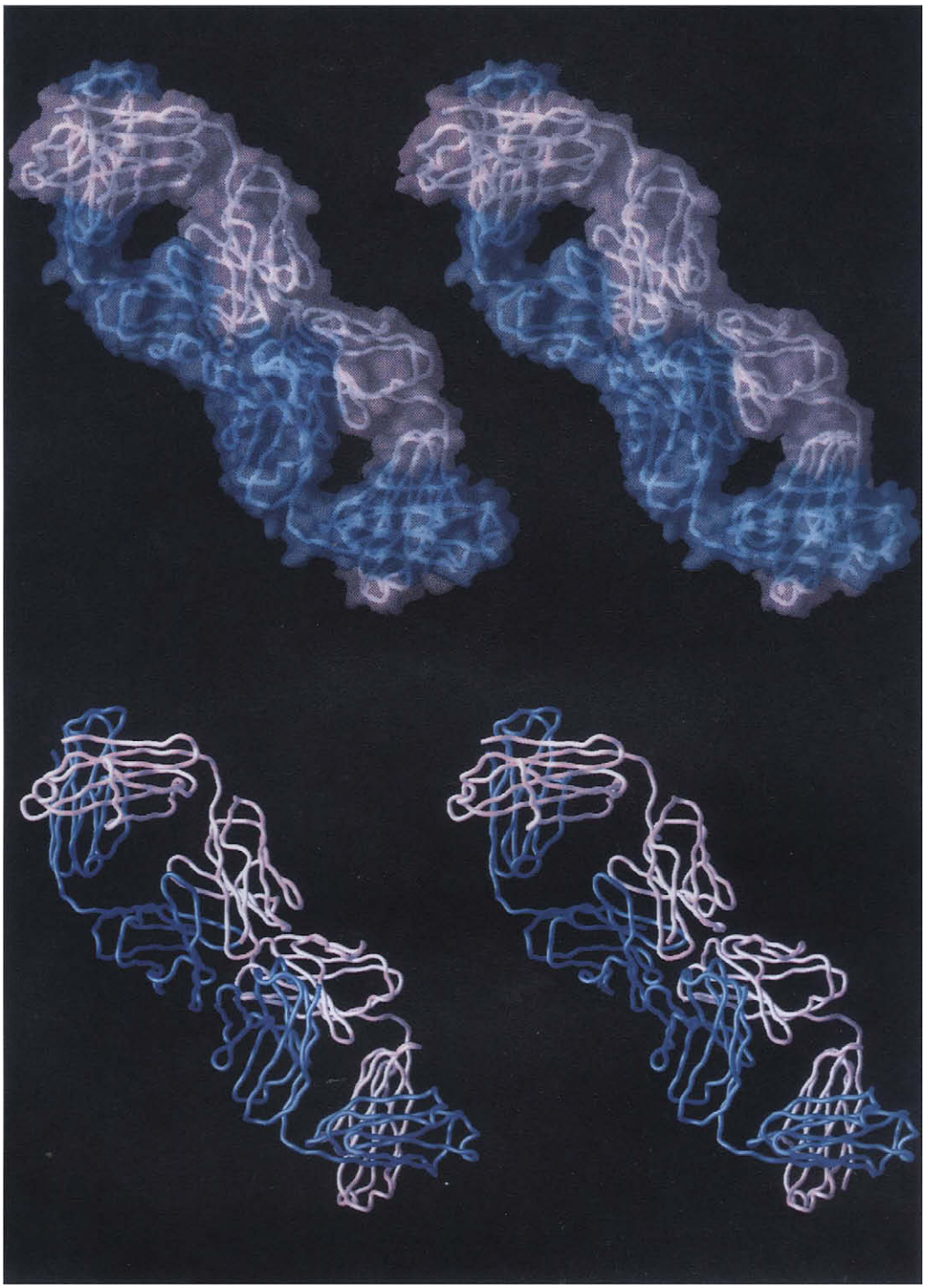


Figure 7. Stereo view of the structure of the two 2-fold related mAb GH1002 Fab molecules reveals "head on" positioning of variable heavy and light chains with respect to the corresponding subunits of the other molecule along the direction of β strands. The Fab molecule is in magenta (light chain) and dark blue (heavy chain). The 2-fold axis crosses between the two Fabs approximately in the plane of the paper (panel **A**). Molecular surface of the Ab3–Ab3 complex demonstrates tight interaction between the two molecules. The orientation and color scheme is the same as in panel A (panel **B**).

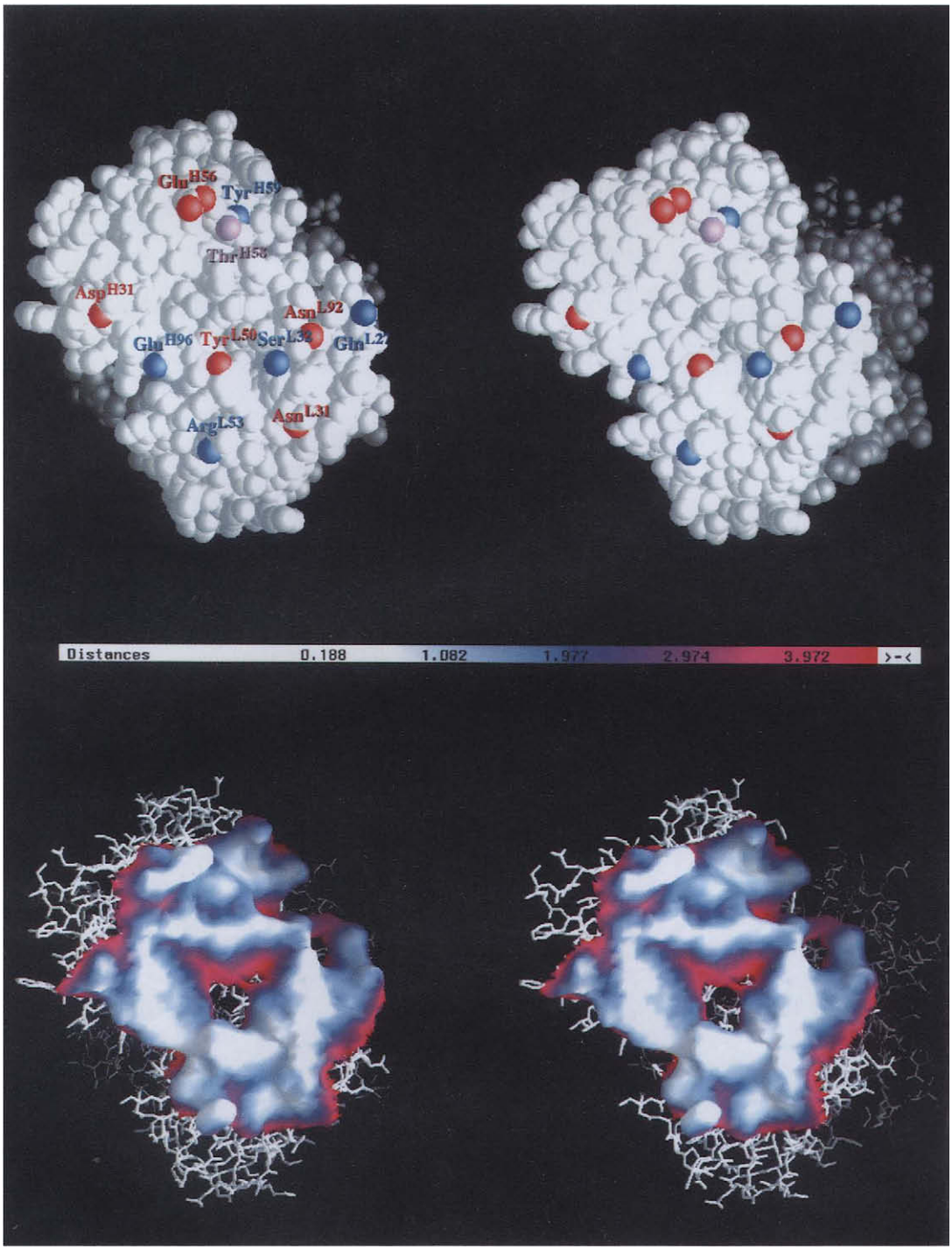


Figure 8. Stereo view of the combining site of anti-anti-Id mAb GH1002. The 2-fold axis of symmetry is approximately in the plane of the paper and vertical. This makes atoms on the left side of the image interact with atoms on the right side of the image of the two identical, 2-fold related, molecules. In panel **A**, all atoms are shown as space filling models. There are 116 van der Waals contacts and 13 probable hydrogen bonds. Twelve groups involved in hydrogen bonding are labeled to facilitate identification of contact points between the two Fabs. The hydrogen bond acceptors are labeled with red and donors with blue. Threonine labeled with magenta is interacting with itself and can be either donor or acceptor. In panel **B**, this region is buried upon complex formation. The surface is color coded according to the distance between two molecules in the complex.

antibody (E5.2). The mimicry is achieved by extensive overlap between the paratope and the idiotope of D1.3. Virtually the same residues of idiotypic D1.3 are involved in binding the original antigen and the anti-idiotope. Furthermore, there is a considerable similarity in the nature of the interactions of the idotypic D1.3 in the antigen–idiotype and the Id–anti-Id complex, and the affinity of the E5.2 for the D1.3 of 1.4×10^5/M approaches the affinity between the antigen and D1.3 of 2.7×10^8/M.

In this study, the interaction between idiotope and anti-idiotope was performed by crystallographic analysis of respective Fv fragments of antibodies. The contacts involve all six CDRs of the two antibodies. Tyr^{L49} is the only residue on both antibodies that is involved in interface contacts. The V_H domains of the two molecules are responsible for a majority of contacts. The V_H domain of the anti-idiotope accounts for 77% of the total contacts with the idiotope. Upon complex formation, 912 Å^2 of the idiotope and 974 Å^2 of the anti-idiotope are buried.

Comparing the structures of the idiotypic antibody D1.3 CDRs in complex with the anti-idiotype with the original antigen, no structural changes were observed. Significant overlap of the paratope and idiotope was evident in the observation of 13 residues on the idiotopic D1.3 interacting both with the anti-idiotopic E5.2 and the antigen. These residues make up 75% (687 Å^2) of the interaction area with the anti-idiotope and 87% (675 Å^2) of the interacting area with the lysozyme. Besides surface complementarity, there are also six out of 12 hydrogen bonds in the D1.3–E5.2 complex that are structurally equivalent to the hydrogen bonds formed in the D1.3–lysozyme complex. This was not observed in the case of the D1.3–E225 complex. Interestingly, solvent molecules contribute to the binding mimicry. The positions of 11 water molecules are conserved to within 1 Å in both the D1.3–E5.2 and D1.3–lysozyme interfaces. Interactions between the two different Id–anti-Id complexes and an antigen–antibody complex are compared in Figure 2 (p. 22), and Table 1.A and B (p. 30).

THE ANTI-FELINE INFECTIOUS PERITONITIS VIRUS SYSTEM

For this idiotope–anti-idiotope system the original antigen was the E2 peplomer, a large glycoprotein, of feline infectious peritonitis virus (FIPV). The E2 peplomer was chosen as an antigen because it is responsible for: a) the binding of virus to plasma membranes of susceptible cells, b) cell fusion, c) induction of cell-mediated cytotoxicity of infected cells, and d) induction of neutralizing antibody (Sturman and Holmes, 1983). This virus, antigenically similar to other coronaviruses, including human coronavirus 229E (Pedersen and Black, 1983), produces a fatal disease in both wild and domestic cats that has defied conventional vaccines (Escobar et al., 1992). A salient observation was that anti-idiotypic antibody 409.5.3 raised against Ab1 730.1.4, when injected back into mice, elicited the production of Ab3 antibodies that had FIP virus neutralizing properties. This indicated that the combining properties of the original epitope were transmitted by the Ab2.

Description of Fabs

Both Fabs have similar elbow angles (138.2° for Fab1 of Ab1 730.1.4 and 141.4° for Fab2 of Ab2 409.5.3). The pseudodyad angle relating V_L and V_H domains is 175° for Fab1 and 178° for Fab2. That relating C_L and C_H1 is 169° and 173° for Fab1 and Fab2, respectively. Both elbow angle and pseudodyad angle values are consistent with values found for other Fabs. Five hypervariable regions of Fab1 can be classified as $L1_2$, $L2_1$, $L3_1$, $H1_1$, and $H2_2$ (*38*). Loop H3 has 12 amino acids and forms a long hairpin structure that extends towards L2 and L1 on the light domain. Two ridges on the surface of the combining site are formed by L1, L3 and H3 on one side and H1 and H2 on the other. Four hypervariable regions of the Fab2 can be classified as belonging to the canonical structures $L2_1$, $L3_1$, $H1_1$ and $H2_4$ (Chothia et al., 1989). Even though loop H1 can be classified as $H1_1$, it has a conformation different from the canonical structure: the side chain of residue Phe 27 is buried within the framework structure instead of residue Phe 29 in the canonical structure model. On the other hand residue Phe 29 of this loop is oriented towards the V_L–V_H domain interface and partially buried. Sequence analysis of the Fab2 indicated that the L1 region belongs to the mouse kappa light chain subgroup IV (Kabat et al., 1991). This loop has 10 residues and main chain atoms of residues 25–27a and residue 33 follow the canonical structure model. Hydrophilic serines between residues 29–31 form a turn that is one residue longer than the turn in the $L1_1$ loop of HyHel-5. Loop H3 of Fab2 has 10 amino acids and forms a broad turn stabilized by a hydrogen bond between the side

chain of Arg 94 and the carbonyl oxygen of Phe 97. The Fab2 CDR's surface is undulating, with no deep grooves as are observed when the antigen is a small molecule (Davies et al., 1990). A shallow cavity exists between the L1, H3 and H2 loops.

Description of the Structure

Two views of the complex are shown in Figure 3 (see p. 23). The two Fab molecules interact by direct juxtaposition of their complementary CDRs. The Fabs are rotated by 61° about the long axis of the complex with respect to one another. The pseudo 2-fold axes relating V_L and V_H domains of the two fragments are nearly collinear, the angle between their axes being 154°. By this rotation, the heavy chain of one molecule is interacting almost entirely with the heavy chain of the other, and similarly light chain with light, but to a much lesser extent. The major axis of the complex is approximately 140 Å.

Idiotope–Anti-Idiotope Interface

Surface representations of the CDRs that form the interface between the two Fabs are shown in Figure 4 (p. 24). There is a striking degree of structural and chemical complementarity between the two, consistent with observations for other antibody–antigen complexes (Davies et al., 1990). Even though water molecules can not be reliably visualized at 2.9 Å resolution, there appear to be no buried waters in the interface. Upon complex formation, 1750 Å^2 are buried. Of this surface, Fab1 accounts for 860 Å^2 and Fab2 for 890 Å^2. These values are significantly greater than the values observed with Fab lysozyme complexes, but very close to the buried surface area in the case of a neuraminidase–Fab complex which conceals about 885 Å^2 for the Fab and 878 Å for the neuraminidase (Tulip et al., 1989). The heavy chain of 409.5.3 dominates the binding and contributes 63% of the surface area of the anti-idiotope buried in the complex. The surface of the Fab1 CDR may be described as slightly concave so that the anti-idiotypic Fab, the antibody in this system, protrudes into it.

Enthalpic contributions in the formation of antibody–antigen complexes arise from van der Waals interactions, hydrogen bonds and salt bridges. Hypervariable loops of two Fabs in contact through van der Waals interactions and hydrogen bonds are: L1 (minor contributor), L3, H1, H2 and H3 of Fab1 (58 atoms) and L1, L3 (minor contributor), H1, H2 and H3 of Fab2 (59 atoms). Loops L1, L2, H1 and H2 on one Fab are in proximity to loops L1, L3, H1 and H3, respectively, on the other molecule (Figure 5). In total, 19 residues of the idiotope and 17 residues of the anti-idiotope participate in 111 long van der Waals interactions <4.11 Å and seven short interactions <3.44 Å (Sheriff et al., 1987) (Table 1.D, pp. 30–31). The interaction is further stabilized by nine hydrogen bonds. The maximum distance between electronegative atoms of a hydrogen bond pair is 3.5 Å.

In both cases residues of Kabat's heavy chain FR1 are involved in the interaction. According to the canonical structure model, these residues belong to the binding site loop H1 (Chothia et al., 1989). Overlap between the hypervariable region H1 deduced from immunoglobulin sequence analysis (Kabat et al., 1991) and the structural loop H1 (Chothia et al., 1989) is only two amino acids. It is probable that the H1 region defined by Chothia et al. (1989) is mainly responsible for interaction with the antigen even though it shows less sequence variation than Kabat's H1 region.

The 730.1.4 idiotope is predominately located on its heavy chain which contributes 71% to the buried area on the idiotope. Since V_H domains usually contribute more surface area in antibody–protein interactions (Davies et al., 1990), it is likely that this domain dominates the interaction between the idiotypic antibody and the antigen. In the structure of the Id–anti-Id complex (anti-lysozyme D1.3 Fab and its anti-idiotypic E225 Fab) (Bentley et al., 1990) both Fabs are centered on V_L domains of opposing Fabs and interact primarily through their V_L domains. On the other hand, the V_H domain of idiotypic Fab D1.3 dominates the interaction with lysozyme. This is the reason that the paratope and the idiotope in the D1.3–E225 system only partially overlap, thus reducing the potential for total molecular mimicry.

In the case of the FIP virus antibody system described here, Ab1 recognizes an epitope on the E2 peplomer of the FIP virus as shown by Western blots (Escobar et al., 1992) even when the E2 protein is completely denatured. This suggests that Ab1 may be specific, not strictly for a structurally unique epitope, but for a sequence unique epitope on the antigen.

It may be noteworthy that a comparison of the sequences of the three light and three heavy chain CDRs with the known sequence of the antigen shows homology in two instances. In both L1 and H1 there

is near identity with sequences of six residues that occur in the antigen. The L1 CDR sequence is Val-Ser-Ser-Ser-Ile-Ser, which differs with the segment on the E2 peplomer beginning at position 276 having sequence Ile-Ser-Ser-Ser-Ile-Ser by the conservative substitution of valine for isoleucine at the first position. Similarly, the H1 loop is Gly-Phe-Thr-Phe-Asn-Asn, which matches the Gly-Phe-Ser-Phe-Asn-Asn sequence of the antigen beginning at residue 1451. Here again, only the conservative change from serine to threonine marks a difference. These two regions of homology on the anti-idiotope provide important contacts with the idiotypic antibody originally produced in response to antigen. Homologous residues on L1 of the anti-idiotope are involved in 36 van der Waals contacts and two hydrogen bonds, and those on H1 form 18 van der Waals contacts and the one hydrogen bond presented in Table 1.D (pp. 30–31) and in Figure 5 (below). The possibility exists, therefore, that antigen sequence information determining specificity may in fact be preserved through the idiotypic antibody and be made to reappear in the structure of the anti-idiotypic response.

ANTI-ANGIOTENSIN II SYSTEM

An exciting structural study dealing with anti-idiotopes was published by Garcia et al. (1992). This work utilized a system in which monoclonal antibody against the angiotensin II (AII), an octapeptide which plays a central role in the regulation of blood pressure in humans and other mammals, was used to obtain anti-idiotypic antibodies. Using these polyclonal anti-idiotypic antibodies as an immunogen, anti-anti-idiotypic antibodies were produced. One of these anti-anti-idiotopes in particular, designated Mab131, was found to bind AII with high

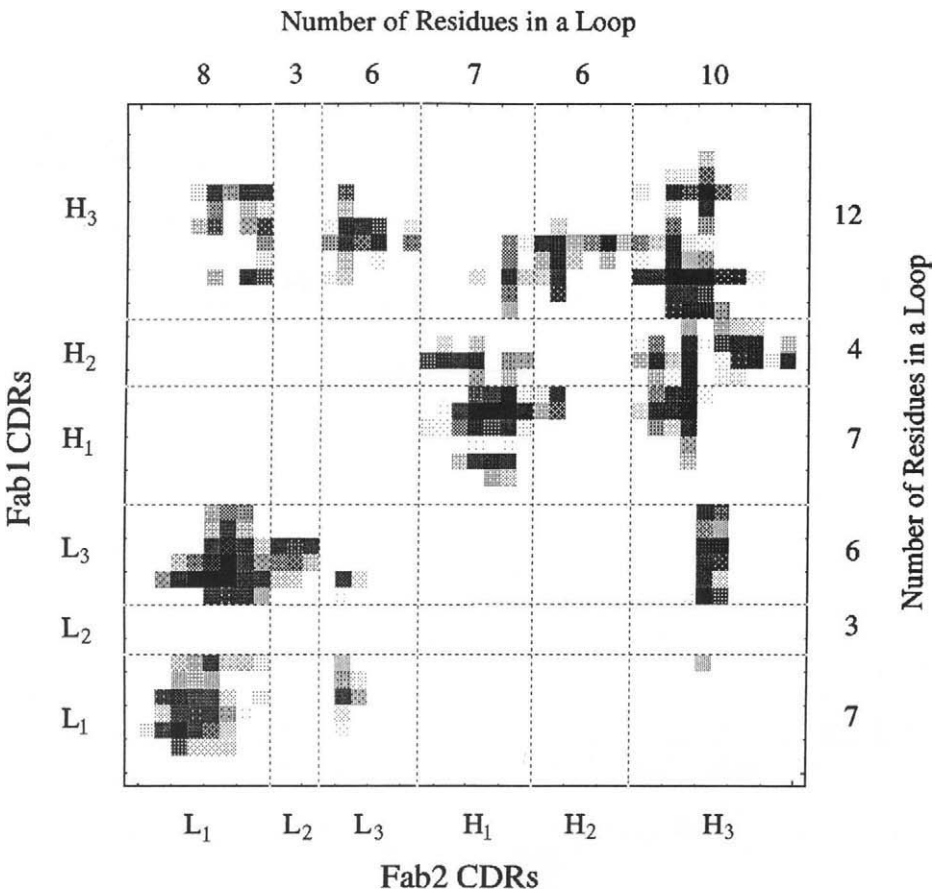


Figure 5. Distance matrix between the idiotope and anti-idiotope (730.1.4–409.5.3) CDRs. Specific loops for each Fab and the corresponding number of residues are indicated. Sizes of hypervariable regions are according to the structural classification of Chothia (*38*), except for loops H3 which follow the convention of Kabat (*39*). Density of the square in the matrix is a function of distance, ranging from darkest <6.5 Å to lightest at 12.5 Å.

Table 1.

A.	D1.3 (Ab1)	E225 (Ab2)				Lysozyme (Ag)
L1	H30	S_L93(2)	Y_L94(10)	F_H102(2)		
	N31	Y_L94(3, 1H)	W_H33(5)	N_H59(1, 1H)	F_H102(4)	
	Y32	W_L50(3)	C_L91(2, 1H)	G_L92(1)	F_H102(10)	VDW
L2	Y50	W_L50(7, 1H)	L_H100(3)	F_H102(4)		1H
	T52	W_H33(3, 1H)	Y_H52(8)			
FR3	S65	S_H55(1)				
	G66	D_H57(5, 1H)				
	S67	D_H57(3, 1H)				
L3	W92	G_L92(2, 1H)	S_L93(1)			VDW
H2	W52	R_L30(7)				VDW
	D54	R_L30(4, 1S)	S_L67(6, 1H)			1H
H3	D100	W_L50(6)				5H
	Y101	R_L30(1)	I_L31(1)	W_L50(5)		1H

B.	D1.3 (Ab1)	E5.2 (Ab2)		Numbers	HEL (Ag)	
L1						
L2	Y49	N_H54 (1H)				
	Y50	Q_H58 (1H)		1	D18	
L3	W92	R_H100b (1H)			—	
	S93	—		2	Q121	
H1	T30	Y_H98 (1H)				
H2	G53	Y_H98 (1H)		3	G117	
	D54	Y_L49 (1H)				
	N56	Q_L100 (1H)				
	D58	Q_L100 (1H)				
H3	E98	Y_H98 (1H)				
	R99	K_H30 (1H)		4	G102	
	Y101	G_H100a (1H)	Y_H98 (1H)	5, 6	Q121,H_2O 749	

C.	YsT9.1 (Ab1)	T91AJ5 (Ab2)	
L1	Y30	S_L28(2H)	
	Y32	W_L90(1H)	SL91(1H)
L2	Y50	Y_H59(1H)	
L3	G91	Y_H100(1H)	
	N92	S_L30(1H)	
H1			
H2	N53	Y_H32(1H)	
	K54	Q_H1(1H)	
	D56	R_L45(1H)	YL48(1H)
	E61	K_L52(1H)	

D.	730.1.4 (Ab1)	409.5.3 (Ab2)			
L1	D28	S_L28(4, 1H)			
L2	R66	S_L28(1)			
L3	H91	Y_H98(2)			
	Y92	S_L28(3)	I_L29(8)	S_L30(18, 1H)	Y_H98(4, 1H)
	S93	S_L31(3)			
	T94	Y_H99(1)			
	F96	Y_H98(4)			
H1	T30	F_H97(3)			

D. *(contd.)*	730.1.4 (Ab1)	409.5.3 (Ab2)			
	N31	F_H97(3)	N_H30(1)		
	Y32	N_H31(3)	F_H97(4)		
	G33	F_H97(2)			
FR2	W50	Y_H98(1)	Y_H99(3)		
	N52	F_H97(6)	Y_H99(1H)		
H2	Y53	Q_H1(9, 1H)	E_H29(12)		
FR3	T59	Y_H99(2)			
H3	Y97	R_H52(1)	N_H52b(2)	L_H96(2)	
	N100	N_L92(3, 1H)			
	Y100a	N_L92(1H)			
	Y100b	L_H96(4)	F_H97(6)	Y_H98(4)	

E.	GH1002 (Ab3)	GH1002 (Ab3) (self complementary interaction)			
L1	Q27	D_H31 (1H)			
	S30	Y_L49 (4)	A_H97(2)		
	N31	Y_L50 (1)	R_L53 (8, 1H)		
	S32	Y_L50 (6, 1H)	A_H97 (1)		
FR1	Y49	S_L30 (4)			
L2	Y50	N_L31 (1)	S_L32(6, 1H)	Y_L50 (15)	R_L53 (1)
	T51	R_L53 (2)			
	R53	N_L31 (8, 1H)	Y_L50 (1)	T_L51 (2)	
L3	N92	Y_H32 (1)	E_H96 (4, 1H)	A_H97 (2)	
	T93	D_H31 (2)	Y_H32 (2)		
	L94	W_H50 (1)	Q_H52 (6)		
HFR1	D31	Q_L27 (1H)	T_L93 (2)		
	Y32	N_L92 (1)	T_L93 (2)		
H1	W50	L_L94 (1)	W_H50 (1)		
	Q52	L_L94 (6)			
H2	T54	A_H61 (1)			
HFR3	E56	T_H58 (1)	Y_H59 (6, 2H)	K_H64 (2)	
	T58	E_H56 (1)	T_H58 (1H)		
	Y59	E_H56 (6, 2H)			
	A61	T_H54 (1)			
	K64	E_H56 (2)			
H3	E96	N_L92 (4, 1H)			
A97	S_L30 (2)	S_L32 (1)	N_L32 (2)		

Notes

L1, L2, L3, H1, H2 and H3 are the CDRs 1, 2, 3, of the light and heavy chains, respectively. FR refers to the framework region. The one letter code is used for amino acids. The numbering of amino acids follows the convention of Kabat (30), except in section A where the sequence of the idiotope E225 and the anti-idiotope D1.3 is numbered sequentially. Van der Waals contacts, where available, are indicated with numbers in parenthesis beside a residue name and number. Hydrogen bonds and salt links are indicated with number and letter H and S, respectively.

A. Contacts between D1.3 (Ab1) and E225 (Ab2). Under the lysozyme column the type of contact between idiotopic D1.3 and the lysozyme is specified according to the D1.3 residues involved. The residues of the D1.3 which are involved in contacts in both Ag–Ab1 and Ab1–Ab2 complexes are underlined in the D1.3 column.

B. Contacts between D1.3 (Ab1) and E5.3 (Ab2). Under the lysozyme column contacting residues between idiotopic D1.3 and the lysozyme are indicated. This contacts are superimposable in space with contacts between D1.3 (Ab1) and E5.3 (Ab2) which involve underlined residues in the D1.3 column. Numbers before the E5.2 column correlate this contacts to the atoms which are involved in forming this conserved contacts labeled in Figure 2.

C. Contacts between YsT9.1 (Ab1) and T91AJ5 (Ab2).

D. Contacts between idiotopic 730.1.4 and anti-idiotopic 409.5.3. Residues of the anti-idiotope 409.5.3 which show sequence homology with the original antigen are underlined.

E. Contacts between two self complementary mAb GH1002 Fab molecules.

affinity (7.3×10^9/M), a value similar to that of the original antibody which had an affinity constant of 3.2×10^9/M. The structure of the anti-anti-idiotypic Mab131 in complex with the angiotensin II was solved at 2.9 Å resolution.

The combining site of the Mab131 has long hypervariable loops, especially L1 and H3. Loops L1 and H2 form a deep cleft between them, while H3 runs in the middle of the cleft and folds back, forming a flat foundation for the combining site. In the complex, 22 residues on five out of six hypervariable loops of the antibody are in contact with the antigen. There is 725 Å^2 of the Fab and 620 Å^2 of the peptide buried in the complex. The peptide predominantly interacts with the heavy chain of the Mab131. Loops L1, L3, H2, and H3 are main contributors to the binding interactions, and contribute 91% of the total buried area. Several polar contacts can be characterized as hydrogen bonds between: AIIArg^2–Mab131^{L31}, AIIHis^6–Mab131Ser^{L91} and Arg^{H50}, carboxy-terminal carboxylate of AII–MabArg^{H52} and Arg^{H50}.

Anti-idiotypic antibodies in this system transmitted information describing the original antigen and elicited Mab131. To probe the possibility that perhaps one CDR loop on the anti-idiotope could have had a conformation similar to the bound AII, the authors searched the crystallographic structural database. Their analysis indicated that backbone atoms of the AII peptide resemble a CDR loop belonging to the canonical structure $L3_1$. Thus, it is possible that the anti-idiotypic antibody in this system has a CDR that resembles angiotensin II and, further, that Ab3 binding to this CDR also binds to the antigen. In this instance, the mimic of the peptide in the Ab2 may be a single hypervariable loop.

ANTI-LIPOPOLYSACCHARIDE A ANTIGEN OF *BRUCELLA ABORTUS* SYSTEM

The murine monoclonal antibody YsT9.1 binds cell wall polysaccharide A antigen of *Brucella abortus* (the causative agent of brucellosis in bovidae and *Homo sapiens*). The polysaccharide antigen is an α1,2-linked polymer of 4,6-dideoxy-4-formamido-α-D-mannopyranose. None of the anti-idiotopic antibodies raised against idiotypic YsT9.1 were found to mimic the antigen, although these Ab2s competed for the antigen binding site of the idiotopic antibody with the polysaccharide antigen. Chemical modification studies suggested that anti-idiotypic antibody T91AJ5 binds to the same site on the idiotope as the antigen. Crystallographic analysis of the complex between a Fab fragment of YsT9.1 and a Fab fragment of T91AJ5 revealed that anti-idiotypic T91AJ5 was unable to carry an internal image of the original antigen because the polysaccharide binding cleft is too narrow and deep to allow comprehensive contact with the Ab2 CDRs (Evans et al., 1994). This makes T91AJ5 antibody a class γ anti-idiotype.

Two molecules forming a complex interact head to head, with the angle between the pseudo 2-fold axis of the variable domain dimers equal to 178°. The two Fabs are rotated approximately 90°, with respect to each other along the long axis of the complex. All inter-Fab contacts in the complex occur through CDRs except for the amino terminus of the heavy chain of the anti-idiotype. Heavy and light chains of each Fab contact both heavy and light chains of the other Fab. There are 12 putative hydrogen bonds at the interface of this complex (Table 1.C, pp. 30–31). Tyrosine residues predominate in the formation of hydrogen bonds, and they also form a large aromatic ring network which spans three of the four variable domains. The total solvent excluded area is 730 Å^2 for the idiotope and 760 Å^2 for the anti-idiotope. Each Fab has two distinct binding surfaces as a result of the groove structure of the idiotope (Figure 6, p. 24). The groove on the idiotypic YsT9.1 segregates light and heavy chains, while the two binding surfaces of the anti-idiotope are shared between heavy and light chains. The antigen binding groove on the YsT9.1 is 20 Å long, 15 Å wide and 10 Å deep. The binding surface of the anti-idiotope does not contact lower sides and the floor of the groove on the idiotope. This depression might be too deep to construct hypervariable loops of the anti-idiotypic antibody which would fill it completely, and this, it appears, is what would be required in order to make an effective mimic for the antigen.

SELF COMPLEMENTARITY OF A MONOCLONAL ANTIBODY GENERATED IN AN IDIOTYPIC CASCADE

Crystallographic analysis of the Fab fragment of the mouse anti-anti-Id mAb GH1002 (Ab3) revealed that it exists in the crystal as dimers related by crystallographic 2-fold axes (Ban et al., 1996). This antibody was elicited with the syngeneic anti-Id mAb MK2-23 (Yang et al., 1993). The latter mAb, which bears

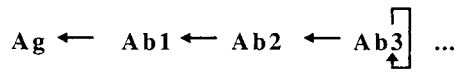
an internal image of the antigenic determinant defined by anti-HMW-MAA mAb 763–774, has been shown to induce anti-HMW-MAA immunity in patients with malignant melanoma (Kusama et al., 1989; Chen et al., 1991; Mittleman et al., 1992; Chen et al., 1993). To characterize the immunological and structural organization of the idiotypic cascade in the HMW-MAA, a panel of anti-anti-Id mAbs was developed from a BALB/c mouse immunized with mAb MK2-23 (Yang et al., 1993). Immunochemical analysis of 11 anti-anti-Id mAbs showed that eight reacted with the immunizing mAb MK2-23 and with HMW-MAA, and three with the immunizing mAb MK2-23 alone. The latter three include mAb GH1002 which binds anti-Id mAb MK2-23 with an association constant of 4×10^9/M and inhibits its binding to the Id mAb 763.74.

Structure of the Fab Fragment

This Fab fragment shares, along with other Fabs whose crystal structures have been determined, a common 3-D fold. The elbow angle of the molecule is 176°. The CDR's surface is undulating with a relatively deep cavity formed by the L2, L3, H1 and heavy chain framework (FR)-3 regions. Five hypervariable regions of the mAb GH1002 can be classified as $L1_2$, $L2_1$, $L3_1$, $H1_1$ (with a Trp instead of Gly at position 26) and $H2_2$ (with a Glu instead of Gly at position 55) (Chothia et al., 1989). A short H3 loop has seven amino acids and has no pronounced features such as bulky residues pointing at the surface of the binding region. This loop is stabilized by a hydrogen bond between residues Asp^{H101} and Glu^{H100}. There is also a salt link formed between Arg^{H94} and Asp^{H101} and Glu^{H100}.

The Packing of the Fab Fragment and Self-Complementary Interactions

Packing analysis revealed a tight interaction between four 2-fold related pairs of Fab fragments in the unit cell (Figure 7, p. 25). Although the combining region of the molecule accounts for a relatively small area of the whole Fab, the head-to-head interactions between the two molecules were responsible for nearly half of the total packing contacts. The crystallographic 2-fold axis bisected the complex between light and heavy chains. mAb GH1002 is crossreactive and, apparently, an auto-anti-anti-Id antibody, as it binds not only the immunizing mAb MK2-23, but also itself. This can be summarized as follows:



The Structure of the Complex

A view of the complex is shown in Figure 7, p. 25. The two Fab molecules interact by direct juxtaposition of their CDRs. The Fab fragments are not noticeably rotated with respect to one another around the long axis of the complex, since their elbow angle axes are nearly parallel with the crystallographic 2-fold axes. The pseudo 2-fold axes relating variable light and variable heavy domains of the two Fab fragments are collinear. Because of this arrangement, the heavy and light chains of one molecule interact principally with the corresponding subunits of another molecule.

The Interface between Self Complementary Anti-anti-idiotopes

Surface representations of the CDRs that form the interface between two Fab fragments are shown in Figure 8, p. 26. The degree of structural and chemical complementarity between the two Fab fragments of mAb GH1002 is comparable with other antibody–antigen complexes (Davies et al., 1990). Upon complex formation, 1103 $Å^2$ of surface area are buried on each Fab fragment. This value is significantly greater than those observed for most other complexes (Sheriff, 1993). The heavy and light chains of mAb GH1002 play a similar role in the binding and each contribute approximately 50% of the total area of the anti-anti-Id mAb in the complex. The surface of the interface may be described as circular with a diameter of approximately 35 Å and a small cavity in the center, presumably occupied by water molecules.

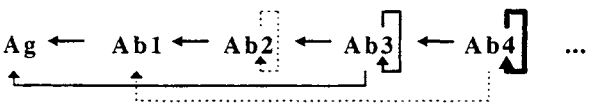
Hypervariable loops of two Fabs in contact through van der Waals interactions and hydrogen bonds are: L1, L2, L3, H1, H2 (minor contributor), heavy chain FR3 and H3 (65 atoms). Loops L1 and L2 on one Fab are in contact with loops L1 and L2, on the other molecule, while heavy chain FR3 provides 17 van der Waals contacts and three hydrogen bonds, binding primarily to the same framework element of the symmetry related molecule. In total, 23 residues of the anti-anti-Id mAb participate in 116 van der Waals interactions <4.11 Å (Table 1.E, pp. 30–31). The interaction is further stabilized by 13 probable hydrogen bonds.

Possible Implications of the Self-Complementary Interaction

The self-complementary interaction of the Fab fragment of mAb GH1002 could be a consequence of crystallization conditions and be coincidental, but there is some evidence that would suggest otherwise. The extensive and intimate contact, judged according to buried surface area, hydrogen bonds, and van der Waals bonds are not consistent with the more tenuous crystal packing interactions usually observed. Interactions are very tight and comparable to those seen in antibody–antigen complexes. In spite of the fact that antibodies and particularly Fab fragments are becoming the most studied family of proteins (Davies and Chacko, 1993; Padlan, 1994), similar self-interaction has never been reported. Furthermore, the original antigen for this anti-anti-antibody is the CDR region of another antibody (anti-idiotypic MK2-23). It is possible that the interaction between the anti-anti-idiotypic mAb GH1002 and the anti-idiotypic MK2-23 involves certain framework regions. These regions are likely to be similar for the two antibodies, and could be the basis for the self-recognition of GH1002 in the crystal.

A possible physiological role is suggested by the observed structural complementarity, though, of course, there may be alternatives. Because it would be immunologically futile to prolong an anti-idiotypic cascade indefinitely, evolution of a cascade toward self-neutralization would provide a mechanism for modulation and ultimate termination. Indeed, isologous interactions based on dyad symmetry are usually the basis for energetically favorable protein–protein interactions in oligomers (Monod et al., 1965; Morgan et al., 1979); Id–anti-Id complexes of Fabs already possess pseudo dyad axes, by virtue of the roughly collinear disposition of Fabs, relating framework portions of the molecules (Bentley et al., 1990; Ban et al. 1994; Evans et al., 1994; Fields et al., 1995), and the only parts of the antibody subject to change in an anti-idiotypic series, and therefore capable of exerting self regulation, are the CDRs.

If this idea is fundamentally correct, then it implies that interacting antibodies downstream in the idiotypic cascade will have increasingly similar surfaces and that self recognition would be enhanced along the cascade as well. In the process, the structural information reflecting the original antigen would gradually be lost. The process could be summarized as follows:



The structure of the complex between anti-Id mAb MK2-23 and anti-anti-Id mAb GH1002 has not been solved yet. It seems likely, however, that the nature of the interaction between these two molecules will be considerably different from that for the Ab3–Ab3 complex. Formation of a 2-fold axis of symmetry between mAb MK2-23 and GH1002 is impossible, and it is likely that the general disposition of the antibodies forming the complex will be different, though some interactions may be similar to those observed in the complex described here.

REFERENCES

Alzari PM, Lascombe M-B, Poljak RJ. Three-dimensional structure of antibodies. Ann Rev Immunol 1988;6:555–580.

Amzel LM, Poljak RJ. Three-dimensional structure of immunoglobulins. Ann Rev Biochem 1979;48:961–997.

Anders EM, Kapaklis-Deliyannis GP, White DO. Induction of immune response to influenza virus with anti-idiotypic antibodies. J Virol 1989;63:2758–2767.

Ban N, Escobar C, Garcia R, Hasel K, Day J, Greenwood A, McPherson A. Crystal structure of an idiotype–anti-idiotype Fab complex. Proc Nat Acad Sci USA 1994;91:1604–1608.

Ban N, Day J, Wong X, Ferrone S, McPherson A. Crystal structure of an anti-anti idiotype shows it to be self-complementary. J Mol Biol 1996;255:617–627.

Baniyash M, Eshhar Z. Anti-anti-IgE idiotypic antibodies mimic IgE in their binding to the Fc_e receptor. Eur J Immunol 1987;17:1337–1342.

Bentley GA, Boulot G, Riottot MM, Poljak RJ. Three-dimensional structure of an idiotope–anti-idiotope complex. Nature 1990;348:254–257.

Bhat TN, Bentley GA, Fischmann T, Boulot G, Poljak RJ. Small rearangement in structures of Fv and Fab fragments of antibody D1.3 on antigen binding. Nature 1990;347:483–485.

Chanh TC, Rappocciolo G, Hewetson JF. Monoclonal anti-idiotope induces protection against the cytotoxisity of the trichothecene mycotoxin T-2. J Immunol 1990; 144:4721–4728.

Chen ZJ, Yang H, Ferrone S. Human high molecular weight melanoma-associated mimicry by mouse antiidiotypic monoclonal antibody MK2-23 characterization of the immunogenicity in syngeneic hosts. J Immunol 1991; 147:1082–1090.

Chen ZJ, Yang H, Liu CC, Hirai S, Ferrone S. Modulation by adjuvants and carriers of the immunogenicity in xenogeneic hosts of mouse anti-idiotypic monoclonal antibody MK2-23, an internal image of human high molecular weight melanoma associated antigen. Cancer Res 1993; 53:112–119.

Chothia C, Lesk AM, Tramontano A, Levitt M, Smith-Gill SJ, Air G, Sheriff S, Padlan EA, Davies D, Tulip WR, Colman PM, Spinelli S, Alzari PM, Poljak RJ. Conformations of immunoglobulin hypervariable regions. Nature (London) 1989;342:877–883.

Dalgleish AG, Kennedy RC. Anti-idiotypic antibodies as immunogens: idiotype-based vaccines. Vaccine 1988;6: 215–220.

Davie JM, Seiden MV, Greenspan NS, Lutz CT, Bartolow TL, Clevinger BL. Structural correlates of idiotopes. Ann Rev Immunol 1986;4:147–165.

Davies DR, Chacko S. Antibody structure. Acc Chem Res 1993;26:421–427.

Davies DR, Metzger HA. Structural basis of antibody function. Ann Rev Immunol 1983;1:87–117.

Davies RD, Padlan AE, Sheriff S. Antibody–antigen complexes. Ann Rev Biochem 1990;59:439–473.

Escobar JC, Kochik SA, Skaletsky E, Rosenberg JS, Beardsley TR. Immunization of cats against feline infectious peritonitis with anti-idiotypic antibodies. Viral Immun 1992;1:71–79.

Evans SV, Rose DR, To R, Young NM, Bundle DR. Exploring the mimicry of polysaccharide antigens by anti-idiotypic antibodies. J Mol Biol 1994;241:691–705.

Fields BA, Goldbaum FA, Ysern X, Poljak RJ, Mariuzza RA. Molecular basis of antigen mimicry by an anti-idiotope. Nature 1995;374:739–742.

Fons GCM, Uytdehaag M, Osterhaus ADME. Induction of neutralizing antibody in mice against poliovirus type II with monoclonal anti-idiotypic antibody. J Immunol 1985;134:1225–1229.

Garcia KC, Ronco PM, Verroust PJ, Brünger AT, Amzel LM. 3-Dimensional structure of an angiotensin-II Fab complex at 3 angstrom – hormone recognition by an anti-idiotypic antibody. Science 1992;257:502–507.

Gaulton GN, Greene MI. Idiotypic mimicry of biological receptors. Ann Rev Immunol 1986;4:253–280.

Greene MI, Nisonoff A, ed. In: The Biology of Idiotypes. New York: Plenum Press, 1984.

Grieder FB, Schultz KT. Anti-idiotypic antibody mimicry of a bluetongue virus neutralizing antigen. J Immunol 1990;144:2627–2631.

Harris LJ, Larson SB, Hasel KW, Day J, Greenwood A, McPherson A. Three dimensional structure of an intact monoclonal antigody for canine lymphoma. Nature 1992;360:369–372.

Jerne NK. Towards a network theory of the immune system. Ann Immunol (Paris) 1974;125C(1–2):373–389.

Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C. Sequences of Proteins of Immunological Interest, 5th ed. Washington, DC: Public Health Service, NIH, 1991.

Kennedy RC, Eichberg JW, Lanford RE, Dreesman GR. Anti-idiotypic antibody vaccine for type B viral hepatitis in chimpanzees. Science 1986;232:220–223.

Kresina TF, Olds GR. Anti-idiotypic antibody vaccine in murine *Schistosomiasis mansoni* comprising the internal image of antigen. J Clin Invest 1989;83:912–920.

Kusama M, Kageshita T, Chen ZJ, Ferrone S. Characterization of syngeneic antiidiotypic monoclonal antibodies to murine anti-human high molecular weight melanoma-associated antigen monoclonal antibodies. J Immunol 1989;143:3844–3852.

Langone J ed. Antibodies, antigens, and molecular mimicry. Meth Enzymol 1989;178:15–19.

Lindenmann J. Speculations on idiotypes and homobodies. Ann Immunol (Paris) 1973;124C(1–2):171–184.

Mariuzza RA, Poljak RJ. The basics of binding: mechanisms of antigen recognition and mimicry by antibodies. Curr Opin Immunol 1993;5:50–55.

McNamara MK, Ward RE, Kohler H. Monoclonal idiotope vaccine against *Streptococcus pneumoniae* infection. Science 1984;226:1325–1326.

Mittelman A, Chen ZJ, Yang H, Wong GY, Ferrone S. Human high molecular weight melanoma-associated antigen (HMW-MAA) mimicry by mouse anti-idiotypic monoclonal antibody MK2-23 – induction of humoral anti-HMW – MAA immunity and prolongation of survival in patients with stage-IV melanoma. Proc Natl Acad Sci USA 1992;89:466–470.

Monod J, Wyman J, Changeux J-P. On the nature of allosteric transitions: a plausible model. J Mol Biol 1965;12: 88–119.

Morgan RS, Miller SL, McAdon JM. The symmetry of self complementary surfaces. J Mol Biol 1979;127:31–39.

Padlan EA. Anatomy of the antibody molecule. Mol Immunol 1994;31:169–217.

Pain D, Murakami H, Blobel G. Identification of a receptor for protein import into mitochondria. Nature (London), 1990;347:444–449.

Pan Y, Yuhasz SC, Amzel M. Anti-idiotypic antibodies: biological function and structural studies. FASEB J 1995;9:43–49.

Pedersen NC, Black JW. Attempted immunization of cats against feline infectious peritonitis, using avitrulent live virus or sublethal amounts of virulent virus. Am J Vet Res 1983;44(2):229–234.

Poskitt DC, Jean-Francois MJB, Turnbull S, MacDonald L, Yasmeen D. Internal image (Ab2β) anti-idiotype vaccines. Theoretical and practical aspects. Vaccine 1991;9:792–796.

Reagan KJ, Wunner WH, Wiktor TJ, Koprowski H. Anti-idiotypic antibodies induce neutralizing antibodies to rabies virus glycoprotein. J Virol 1983;48:660–666.

Rodkey LS. Studies of idiotypic antibodies. Production and characterization of autoantiidiotypic antisera. J Exp Med 1974;139:712–720.

Sacks DL, Esser KM, Sher A. Immunizations of mice against african Trypanosomiasis using anti-idiotypic antibodies. J Exp Med 1982;155:1108–1119.

Schreiber JR, Nixon KL, Tosi MF, Pier GB, Patawaran MB.

Anti-idiotype-induced, lipopolysacharide-specific antibody response to *Pseudomonas aeruginosa*. J Immunol 1991;146:188–193.

Sheriff S. Some methods for examining the interactions between two molecules, Immunomethods 1993;3:191–196.

Sheriff S, Hendrickson WA, Smith JL. Structure of Myohemerythrin in the azidomet state at 1.7/1.3 Å resolution, J Mol Biol 1987;197:273–296.

Sturman LS, Holmes KV. The molecular biology of coronaviruses. Adv Virus Res 1983;28:35–112.

Tulip WR, Varghese JN, Webster RG, Air GM, Laver WG, Colman PM. Cold Spring Harbor Symp Quant Biol 1989;54:257–263.

Velge-Roussel F, Valwaerde C, Grzych JM, Auriault A, Capron A. Protective effects of anti-idiotypic IgE antibodies obtained from an IgE monoclonal antibody specific for a 26-kilodalton *Schistomosoma mansoni* antigen. J Immunol 1989;142:2527–2730.

Williams WV, Weiner DB, Kieber-Emmons T, Greene MI. Antibody geometry and form: three-dimensional relationship between anti-idiotypic antibodies and external antigens. Trends Biotechnol 1990;8:256–263.

Yang H, Chen ZJ, Kageshita T, Yamada M., Ferrone S. Idiotypic cascade in the human high molecular weight melanoma-associated antigen system – fine specificity and idiotypic profile of anti-anti-idiotypic monoclonal antibodies. Eur J Immunol 1993;23:1671–1677.